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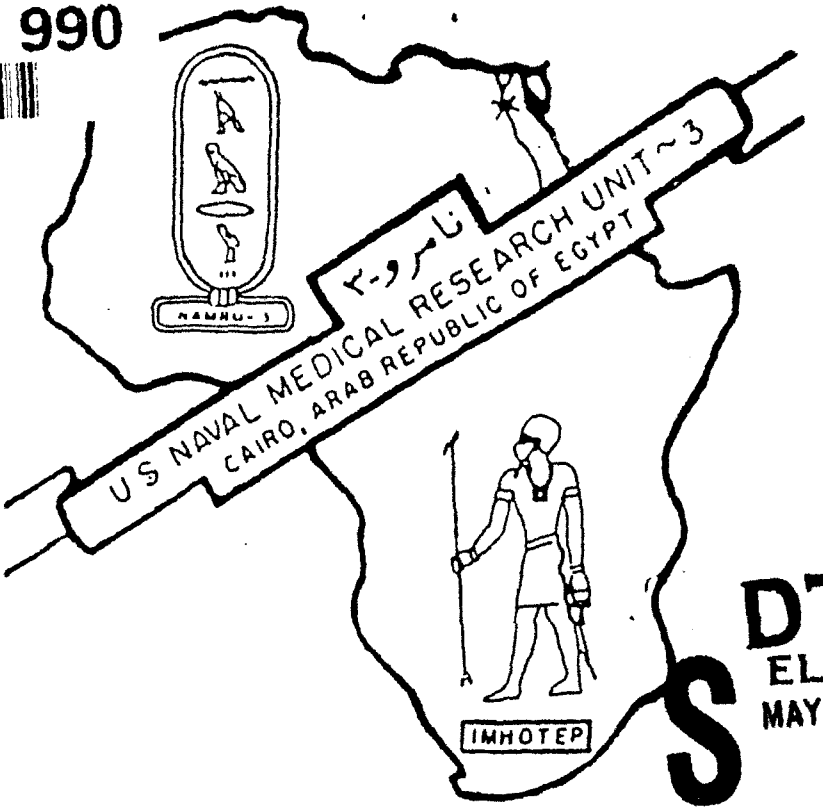

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THE ROLE OF IGG ANTIBODIES FROM IRRADIATED CERCARIA-IMMUNIZED RABBITS IN THE PASSIVE TRANSFER OF IMMUNITY TO SCHISTOSOMA MANSONI-INFECTED MICE

BY

Beverly L. Mangold and David A. Dean

U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3
(CAIRO, ARAB REPUBLIC OF EGYPT)
PSC 452, BOX 5000
FPO AE 09835-0007

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THE ROLE OF IgG ANTIBODIES FROM IRRADIATED CERCARIA–IMMUNIZED RABBITS IN THE PASSIVE TRANSFER OF IMMUNITY TO *SCHISTOSOMA MANSONI*–INFECTED MICE

BEVERLY L. MANGOLD AND DAVID A. DEAN
U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Abstract. Antibodies of the IgG subclass isolated from the sera of rabbits immunized with cercariae subjected to 50 kilorads of gamma irradiation passively provided partial immunity against *Schistosoma mansoni* challenge in C57Bl/6J mice. These mice exhibited reductions in adult worm burdens of 43–61% compared with recipients of normal rabbit antibodies. Passively transferred IgG antibodies were most effective when given 4–7-days postchallenge; they were less effective when given just before challenge, and were totally ineffective when given 15 days postchallenge. It was also shown that the Fc portion of the IgG molecule was important for passive transfer of immunity. Finally, we observed that although some antibodies from irradiated cercaria–immunized rabbits recognized keyhole limpet hemocyanin (KLH), these KLH cross-reacting antibodies were not necessary for successful passive transfer of immunity. Antibodies from a KLH-immunized rabbit also failed to passively protect mice.

Our laboratory has previously shown that partial immunity against *Schistosoma mansoni* infection can be passively transferred to C57Bl/6 mice with IgG antibodies isolated from the serum of mice multiply immunized with cercariae irradiated with 50 kilorads of gamma irradiation.[1] In addition, Bickle and others have demonstrated the effectiveness of serum from irradiated cercaria–immunized rabbits in partially protecting mice against an *S. mansoni* challenge.[2] The use of rabbits as antibody donors has the advantage that large amounts of serum can be collected from a relatively small number of animals, which can be repeatedly boosted. In the current study, we attempted to confirm the effectiveness of a heterologous passive transfer system and to further elucidate the requirements of passive transfer of immunity. Our results confirm the finding of Bickle and others that serum from irradiated cercaria–immunized rabbits can passively protect mice. We also extend these findings by demonstrating that 1) IgG antibodies are specifically involved in protection, 2) the timing of injection is crucial, and 3) the Fc portion of the antibody molecule is necessary for passive protection.

Grzych and others have shown that *S. mansoni* shares a protective carbohydrate epitope with keyhole limpet hemocyanin (KLH); rats immunized with KLH exhibited reductions of 50–75% in the number of adult worms recovered compared with control rats, and naive rats receiving serum from KLH-immunized rats were partially protected (48% worm reduction) against an *S. mansoni* challenge.[3] In light of these studies, we investigated whether antibodies from irradiated cercaria–immunized rabbits reacted with KLH and, if so, whether they had a role in passive protection. We found that serum from irradiated cercaria–immunized rabbits contained antibodies that recognized KLH. However, neither these cross-reacting antibodies nor antibodies from a KLH-immunized rabbit were found to be important in passive immunity in this model.

MATERIALS AND METHODS

Parasite

A Puerto Rican–derived strain of *S. mansoni* maintained in *Biomphalaria glabrata* was used.[4]

Serum donors

Male New Zealand white (NZW) rabbits (Hazleton Research Animals, Denver, PA) served as donors of serum. Rabbits were immunized by percutaneous exposure to cercariae subjected 1 hr earlier to 50 kilorads (1, 227 rad/min) of gamma-radiation from a ^{137}Cs source. They received approximately 5,000 irradiated cercariae for the

first immunization and 10,000–12,000 irradiated cercariae for subsequent immunizations, which were given three months apart. At each immunization, rabbits were anesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg of body weight; Vetalar; Parke-Davis, Morris Plains, NJ) and acepromazine (0.5 mg/kg body weight; Ayerst, New York, NY), and exposed to half the irradiated cercariae on shaved abdominal skin and half on one of the ears (right and left ears being alternately used as immunization sites). Blood was collected from the ear of anesthetized rabbits (Innovar; 0.2 ml/kg; Pitman-Moore, Washington Crossing, NJ) three weeks after immunization and again at 5–6 weeks after immunization. Serum was prepared as previously described.[1] Six immunized rabbits were used as donors. Normal serum was collected from several age-, strain-, and sex-matched control rabbits. The experiments reported herein were conducted according to the principles set forth in the current edition of the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

Antibody fractionation

The IgG portions of immune and normal rabbit sera were fractionated by Protein A-Sepharose 4B affinity chromatography, as previously described.[1] The whole immunoglobulin fraction was prepared by precipitating serum twice with ammonium sulfate, pH 7.2 (50% final concentration).[1] Protein concentrations of purified immunoglobulins were determined spectrophotometrically (assuming 1 optical density unit at 280 nm = 0.7 mg/ml of purified immunoglobulins).[5]

Analysis of resistance

Female C57Bl/6 mice (Jackson Laboratory, Bar Harbor, ME) were age-matched in each experiment, but overall, groups of mice were between six- and 10-weeks old when challenged. Restrained, unanesthetized mice were challenged percutaneously by a 1-hr tail exposure to approximately 150 cercariae (exact numbers are given in the text).

Adult worms were recovered from killed animals six weeks after challenge infection by a modification[6] of the hepatic portal perfusion technique.[7]

Resistance to challenge infection was expressed as the percentage reduction in the number of adult worms recovered from immune antibody recipients compared with normal antibody recipients, and was calculated as previously described.[1] The resistance data were analyzed statistically by the two-tailed Student's *t*-test. *P* values less than 0.05 indicated significant differences among the groups compared.

Preparation of F(ab')$_2$ fragments

Rabbit IgG was digested with pepsin (Sigma, St. Louis, MO) according to standard procedures.[8] The sample was then fractionated on a Protein A-Sepharose 4B column to remove any undigested IgG. The non-bound fraction was concentrated by ultrafiltration (10,000 MW cut-off filters: Amicon; Danvers MA), and then fractionated on a Sephadex G-50 column equilibrated with 0.15 M phosphate-buffered saline (PBS), pH 7.2 and 0.02% sodium azide. The fragments were collected in the void volume, concentrated by ultrafiltration, dialyzed extensively against PBS, and stored at −70°C until used.

To facilitate testing of the effectiveness of pepsin digestion, 1 ml of rabbit IgG anti-sheep red blood cell hemolysin (Cappel, West Chester, PA) was added to 40 ml of serum from irradiated cercaria-immunized rabbits prior to isolation of IgG by Protein A-Sepharose 4B chromatography. Both undigested and pepsin-digested sera were then tested for their ability to agglutinate sheep red blood cells (SRBC) and to support complement-mediated lysis of SRBC.

Hemagglutination. Sheep red blood cells were washed three times in physiologic saline and diluted to a 1% suspension in veronal-buffered saline (VBS), pH 7.4 (5 mM Na 5, 5-diethylbarbituric acid, 142 mM NaCl, 200 mM $MgCl_2 \cdot 6H_2O$, 30 mM $CaCl_2$) plus bovine serum albumin (BSA, crystalline; Calbiochem, San Diego, CA)) at a concentration of 1 mg/ml (VBS/BSA). Rabbit IgG and pepsin-generated F(ab')$_2$ preparations were diluted in VBS/BSA to an initial concentration of 1 mg/ml. Twenty-five microliter aliquots of antibodies were serially diluted two-fold in VBS/BSA in the wells of round-bottom microhemagglutination plates, and 25 μl of 1% SRBC was added to each well. The plates were sealed and incubated for 1 hr at room temperature. The titers reported are the highest dilutions to give clear agglutination of SRBC.

Complement-mediated lysis of SRBC. Rabbit antibody fractions and SRBC were diluted in

TABLE 1

Passive transfer to mice of protection against Schistosoma mansoni *infection with IgG isolated from irradiated cercaria–immunized rabbits*

Experiment*	Antibody	Worm burden, mean ± SD (no. of mice)	% protection	P
1	—	80.2 ± 11.3 (17)	—	
	Normal IgG	84.7 ± 16.9 (6)	—	
	Immune IgG (rabbit M2)	46.3 ± 10.3 (7)	45.3	<0.001
	Immune IgG (rabbit M6)	33.0 ± 7.1 (6)	61.0	<0.001
	Immune IgG (rabbit M8)	34.7 ± 7.2 (7)	59.0	<0.001
2	Normal IgG	65.7 ± 2.6 (12)	—	
	Immune IgG (pool of M2, M6, and M8)	29.5 ± 4.2 (6)	55.1	<0.001

* All mice received an injection of protein A–purified IgG equivalent to 1 ml of serum on day 4 and day 7 postchallenge. IgG was obtained from normal rabbits or rabbits bled three weeks after a third immunization with 50-kilorad irradiated–cercariae and was administered by intravenous (experiment 1) or intraperitoneal (experiment 2) injection. Mice were challenged with 157 (experiment 1) or 135 (experiment 2) penetrating cercariae.

VBS/BSA and added to the plates, as above. In addition, 50 μl of guinea pig complement (Cappel) diluted 1:20 in VBS/BSA or VBS/BSA alone was added to the wells. The plates were sealed and incubated 1 hr at 37°C. The titers reported are the highest dilution to give complete lysis of SRBC.

Enzyme-linked immunosorbent assay (ELISA)

Keyhole limpet hemocyanin (*Megathura crenulate*; MW = 3–7 × 10^6; Calbiochem) was used to coat polystyrene flat-bottom 96-well microtiter plates (Immunlon 2; Dynatech Laboratories, Alexandria, VA) at a concentration of 0.2 μg/well. A standard ELISA protocol[8] was used with the following modifications: washing buffer (36 mM boric acid, 158 mM NaCl, 0.05% Tween-20; 0.01% thimerosal, pH 8) was used in place of PBS and all antibodies were diluted in washing buffer plus 0.5% BSA. Horse radish peroxidase–conjugated goat anti-rabbit IgG (1:4,000 dilution; Tago, Inc., Burlingame, CA) was used as secondary antibody. The plates were developed with ABTS substrate (Kirkegaard and Perry, Gaithersburg, MD) and read after 5 min with a Micro ELISA Reader MR-580 (Dynatech Laboratories) at an optical density of 405 nm.

Rabbit immunization with KLH

A NZW male rabbit was immunized twice with KLH in Ribi adjuvant (0.25 mg of monophosphoryl lipid A, 0.25 mg of trehalose dimycolate, 0.25 mg of cell wall skeleton, 0.02 ml of hexamethyl-tetracosahexane, and 0.002 ml of Tween 80/injection; Ribi ImmunoChem Res Inc., Hamilton, MT). For each immunization, the rabbit received 100 ug of KLH in adjuvant distributed equally into three sites: 1) intramuscular injection in the right hind quarter, 2) intramuscular injection in the left hind quarter, and 3) subcutaneous injection in the neck area. Immunizations were given two months apart. Serum for transfer was collected two weeks after the second immunization.

KLH affinity chromatography

Keyhole limpet hemocyanin (70 mg) was coupled to CNBr-activated Sepharose 4B (4 g of powder; Pharmacia, Piscataway, NJ) according to protocol provided by the manufacturer.

The absorption of irradiated cercaria–immunized rabbit serum was performed as follows. An affinity column of KLH-Sepharose was equilibrated with 0.14 M sodium phosphate buffer, pH 8.0. Immune rabbit serum was passed slowly over the column, and the unbound fraction was precipitated with ammonium sulfate, pH 7.2,[1] extensively dialyzed against PBS, and stored at −70°C until used. Unabsorbed immune and normal sera were precipitated with ammonium sulfate by the same procedure.

RESULTS

In the initial experiments, IgG antibodies isolated from rabbit sera collected three weeks after the third immunization with irradiated cercariae were tested for their ability to transfer passive protection against *S. mansoni* infection to recipient mice (Table 1). In both experiments, mice received two injections of IgG, at four days and seven days postchallenge. These times were selected because previous studies with homologous

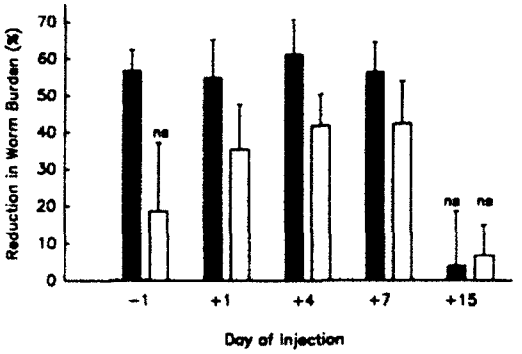

FIGURE 1. Percent reduction in worm burdens in mice receiving 4 mg **(closed bar)** or 1 mg **(open bar)** of IgG from rabbits bled three weeks after the fourth immunization with irradiated cercariae of *Schistosoma mansoni*. Worm burdens were compared with those of mice receiving IgG from unimmunized rabbits. Values are the mean + SD. The reductions at days +1, +4, and +7 at both doses and at day −1 at a dose of 4 mg/ml were highly significant ($P < 0.001$). ns = not significant ($P > 0.05$).

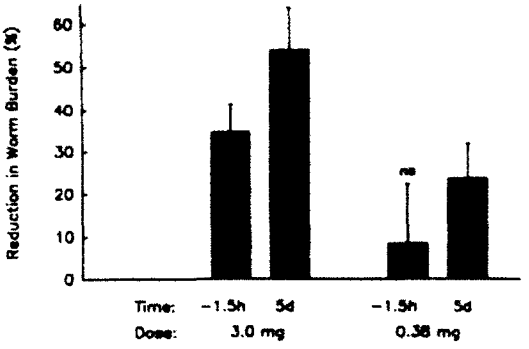

FIGURE 2. Effect of dose and time of injection of rabbit immunoglobulin on transfer of resistance. Mice received ammonium sulfate–precipitated total immunoglobulins from rabbits immunized with irradiated cercariae of *Schistosoma mansoni* or from unimmunized normal rabbits. Values are the mean + SD. The reductions at −1.5 hours (h) and 5 days (d) at a dose of 3 mg and at 5 d at a dose of 0.38 mg were highly significant ($P < 0.001$). ns = not significant ($P > 0.05$).

serum transfer to mouse recipients had shown that serum administered around the time of schistosome lung-phase migration transferred optimal levels of protection.[1] In the first experiment, IgG fractions isolated from three rabbits were tested separately. Mouse recipients of IgG from all three rabbits exhibited highly significant reductions in adult worm burdens compared with recipients of IgG from normal rabbits or no IgG; levels of protection transferred were 45.3%, 59.0%, and 61.0%, respectively. In the second experiment, IgG isolated from these same rabbits was pooled and retested. A comparable level of protection (55.1%) was observed. Although not formally tested in the same experiment, it would appear that the intravenous and intraperitoneal routes of IgG injection are equally effective in transferring protection.

Effect of time and dose of IgG transfer on protection

The transfer efficiency of a single injection of IgG (4 mg/mouse or 1 mg/mouse) administered at various times relative to the time of challenge infection was examined (Figure 1). When the higher dose of IgG was used, significant levels of between 55% and 61% reduction in worm burden ($P < 0.001$) were passively transferred if the antibodies were administered anytime from the day prior to challenge to seven days postchallenge; there was no statistical difference among the reductions seen in the mice receiving antibodies on days -1, 1, 4, and 7.

A slightly different pattern was observed when the amount of IgG was reduced four-fold. In this case, the levels of protection transferred when the immune IgG was administered on days 1, 4, and 7 were all significant, but were approximately one-third lower ($P < 0.05$) than those observed on the same days with the higher dose of IgG. In contrast, 1 mg of immune IgG/mouse given on day −1 no longer transferred a significant level of protection. Irrespective of the dose, immune IgG given 15 days postchallenge was totally ineffective in transferring protection.

The effect of time and dose of Ig transfer was

TABLE 2

Hemagglutination and complement lysis titers

IgG*	Hemagglutination titer†	Complement lysis titer‡
Anti-Sm plus anti-SRBC	240	160
Pepsin-digested anti-Sm plus anti-SRBC	240	<10
Anti-Sm	<15	<10

* IgG from a rabbit multiply immunized with irradiated *Schistosoma mansoni* cercariae (anti-Sm), with and without the addition of 1.40 volume of commercial rabbit IgG anti-sheep red blood cell hemolysin (anti-SRBC), was purified by protein A-Sepharose 4B affinity chromatography. Pepsin digestion of purified IgG was performed as described in the Materials and Methods.

† Reciprocal of highest dilution giving clear agglutination of SRBC.

‡ Reciprocal of highest dilution giving complete lysis of SRBC.

TABLE 3

Comparison of efficacy of intact IgG and F(ab')$_2$ fragments in passively transferring protection

Experiment*	Antibody transferred†	Worm burden, mean ± SD (no. of mice)	% protection‡	P
1	None	76.3 ± 11.9 (12)	—	
	Normal IgG	71.5 ± 10.3 (10)	—	
	Immune IgG	42.5 ± 7.2 (10)	43.1	<0.001
	Normal F(ab')$_2$	77.2 ± 8.8 (5)	—	
	Immune F(ab')$_2$	76.1 ± 16.0 (7)	−1.0	>0.05
2	None	62.3 ± 10.6 (9)	—	
	Normal IgG	60.7 ± 15.0 (6)	—	
	Immune IgG	34.3 ± 10.3 (8)	44.4	<0.001
	Immune F(ab')$_2$	66.8 ± 9.8 (6)	−8.3	>0.05

* Mice were infected with 165 (experiment 1) or 148 (experiment 2) penetrating cercariae.

† Immune denotes that original serum was collected from rabbits three weeks after the fifth immunization with irradiated cercariae. Each mouse received an intraperitoneal injection of 4.5 mg of antibodies on days 4 and 7 postinfection.

‡ Calculated using the average worm burden of all control mice (no antibody and normal antibody recipients), which was 74.7 ± 10.7 (mean ± SD) for experiment 1 and 61.7 ± 12.0 for experiment 2.

retested in a second experiment (Figure 2). In this instance, mice received 3.0 or 0.38 mg of total Ig 1.5 hr prior to challenge infection or five days postchallenge. Significantly more protection was transferred at both doses if the immunoglobulin was administered five days postchallenge rather than near the time of challenge ($P < 0.01$ for 3 mg and $P < 0.05$ for 0.38 mg). Thus, in both experiments it would appear that when the timing of the Ig administration coincided with the lung phase of schistosome migration, the level of protection passively transferred was as high as, and generally higher than, when the Ig was administered at the time of challenge.

Comparison of transfer efficiency of IgG versus pepsin-generated F(ab')$_2$ fragments

The transfer efficiency of pepsin-generated F(ab')$_2$ fragments was compared with that of intact IgG molecules to determine whether the Fc portion of the antibody molecule was necessary to interact with some component(s) of the effector system for immune elimination of the schistosomes, or alternatively, whether binding by the divalent antigen binding portion of the molecule was sufficient.

To facilitate testing of the effectiveness of pepsin digestion, serum from irradiated cercaria–immunized rabbits was combined with a small amount of rabbit IgG anti-SRBC hemolysin prior to isolation of IgG by Protein A-Sepharose 4B affinity chromatography and subsequent pepsin digestion. Both undigested and pepsin-digested preparations were tested for their ability to agglutinate SRBC (an indicator of functional divalent antigen binding activity) and to support complement-mediated lysis of SRBC (an indicator of a functional Fc) (Table 2). Undigested IgG was able to both agglutinate SRBC and support complement-mediated lysis. In contrast, pepsin-digested material was able to agglutinate SRBC as efficiently as intact IgG, but could not support complement-mediated lysis, this being consistent with F(ab')$_2$ activity. Thus, pepsin digestion appears to have successfully cleaved off the Fc portion of the IgG while maintaining F(ab')$_2$ activity.

We examined the abilities of intact IgG and F(ab')$_2$ fragments to passively protect mice (Table 3). In two experiments, it was observed that mice receiving intact IgG from immunized rabbits had a highly significant (43–44%) reduction in the number of adult worms recovered relative to recipients of IgG from normal rabbits or to mice receiving no IgG. In contrast, mice receiving F(ab')$_2$ fragments from immunized rabbits exhibited no reductions in worm burden. Thus, it would appear that the Fc portion of the IgG molecule is necessary for successful passive transfer of protection.

Evaluation of anti-KLH activity in sera of irradiated cercaria–immunized rabbits

In light of the evidence that *S. mansoni* shares a protective carbohydrate epitope with KLH,[3] we investigated whether antibodies from irradiated cercaria–immunized rabbits contained anti-KLH activity. Results from an ELISA using KLH as the antigen indicated that irradiated cer-

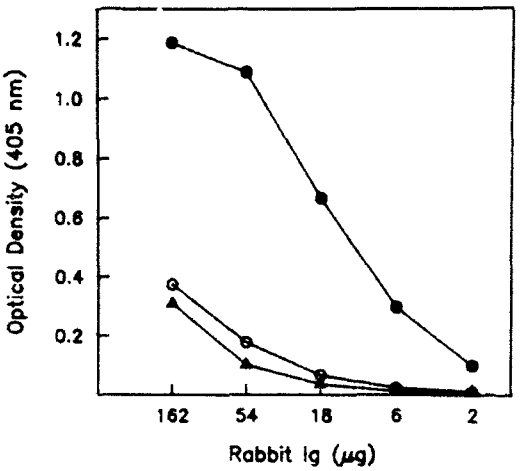

FIGURE 3. Titration of antibodies against keyhole limpet hemocyanin (KLH) as measured by an enzyme-linked immunosorbent assay. Microtiter wells were coated with 0.2 μg of KLH. The wells were incubated with serial dilutions of immunoglobulin from unimmunized rabbits (△), from irradiated cercariae–immunized rabbits (●), or KLH-absorbed immunoglobulin from irradiated cercariae–immunized rabbits (○).

caria–immunized rabbit Ig does recognize an epitope on KLH (Figure 3).

Having identified an anti-KLH activity in immune rabbit serum, we next attempted to determine whether this activity was associated with protection. To test this, we absorbed immune rabbit Ig against KLH by affinity chromatography. The absorption was successful as judged by ELISA analysis (Figure 3); absorbed Ig was indistinguishable from normal Ig. Next, we passively transferred both the absorbed and unabsorbed preparations to determine their protective activities. It was found that immune Ig absorbed against KLH was as effective as unabsorbed immune Ig in passively transferring protection (Table 4).

TABLE 5

Efficacy of rabbit anti–keyhole limpet hemocyanin (KLH) Ig in passively protecting mice from Schistosoma mansoni *infection*[a]

Antibody transferred	Worm burden, mean ± SD (no. of mice)
None	72.5 ± 13.0 (13)
Normal Ig	71.8 ± 6.7 (8)
Anti-KLH Ig	72.1 ± 14.2 (10)

[a] Rabbit M10 was immunized two times with 100 μg of KLH in Ribi (Hamilton, MT) adjuvant; immunizations were given two months apart. Serum was collected two weeks after the second immunization and total Ig was isolated by ammonium sulfate precipitation. Mice received 10 mg of Ig subcutaneously one day and four days postinfection with 157 cercariae.

The above experiment demonstrated that the anti-KLH activity in irradiated cercaria–immunized rabbit serum is not necessary for passive transfer of protection. It did not, however, rule out the possibility that anti-KLH antibodies can passively protect. To test this, we hyperimmunized a rabbit against KLH to be used as a source of antibodies for passive transfer. Serum isolated from the donor rabbit was shown to have a very high titer of anti-KLH antibodies by ELISA (i.e., significant activity was still detected at a serum dilution of 3×10^5). However, Ig isolated from this rabbit was unable to passively protect mice against an *S. mansoni* infection (Table 5).

DISCUSSION

The results presented here demonstrate that IgG antibodies obtained from sera of rabbits multiply immunized with 50-kilorad–irradiated *S. mansoni* cercariae are able to passively transfer high levels of resistance against an *S. mansoni* challenge infection to naive mice (Table 1). The timing of the passive transfer was shown to be critical. Immune IgG antibodies administered during the first week of infection were effective in transferring protection, while IgG given 15

TABLE 4

Efficacy of keyhole limpet hemocyanin (KLH)–absorbed irradiated cercaria–immunized rabbit Ig in passively protecting mice from Schistosoma mansoni *infection*

Antibody transferred[a]	Worm burden (mean ± SD)	% protection	*P*
Normal	73.7 ± 4.7	–	
Immune	32.1 ± 3.8	56	<0.001
KLH-absorbed immune	36.8 ± 4.2	50	<0.001

[a] Mice received an intraperitoneal injection of 5 mg of total Ig on day 4 postchallenge (8 mice/group). Ig was isolated by ammonium sulfate precipitation of serum from an unimmunized rabbit (normal) or a rabbit bled three weeks after the sixth immunization with irradiated cercariae (immune). Immune Ig was also absorbed over a KLH-Sepharose 4B column (KLH-adsorbed immune).

days postchallenge was totally ineffective (Figure 1). By 15 days postchallenge, schistosome migration to the liver in mice is largely complete.[9] Our previous parasite transfer studies have demonstrated that schistosomes are sensitive to immune-dependent elimination through the lung phase of migration, but become totally insensitive to immune elimination once they reach the liver.[10, 11] Thus, the inability of immune IgG to transfer protection on day 15 is probably due to the fact that at this time, the majority of schistosomes have migrated from the lungs to the liver, where they are no longer sensitive to immune elimination.

It was also observed that antibodies were less effective in transferring protection when administered at the time of infection than when administered during the lung phase of migration (Figures 1 and 2). This finding is consistent with our previous passive transfer study, in which it was shown that levels of protection transferred to mice with serum from irradiated cercaria–immunized mice were as good or better when the serum was given seven days postchallenge compared with the day prior to challenge.[1] It is also consistent with our previous findings from autoradiographic migration tracking[9] and parasite transfer[10] studies, which demonstrated that the lung stage schistosomulum is the major target of immune-dependent elimination in irradiated cercaria–immunized mice.

The observation that partial protection can be passively transferred to mice with IgG isolated from irradiated cercaria–immunized rabbits compares favorably with our previous finding of passive transfer to mice with homologous irradiated cercaria-immunized mouse serum or IgG.[1] Indeed, the heterologous transfer system (rabbit to mouse) gave consistently higher levels of protection than the homologous system (mouse to mouse). When optimal transfer conditions were used (i.e., transfer during the first week of infection with 3 mg or more of immunoglobulins), the mean level of protection transferred with rabbit immunoglobulins in 11 experiments was 54 ± 1.9% (mean ± SEM) (range 43–61%). These levels are comparable with those of Bickle and others,[2] who demonstrated that whole sera (adsorbed against mouse red blood cells) from NZW rabbits multiply immunized with irradiated cercariae were able to transfer significant levels of protection when transferred to mice around the time of challenge (43.4 ± 4.0%, range 34–69%) or 5–6 days postchallenge (41.0 ± 9.3%, range 31–56%) (our calculations of averages). In a similar study, mice injected with 400 μg of Protein A–purified IgG from 60-kilorad–irradiated cercaria–immunized NZW rabbits exhibited reductions in adult worms of 21.6–28.4%.[12]

To evaluate the effector mechanisms involved in the passive protection of mice, we compared the ability of immune intact IgG antibodies and pepsin-generated $F(ab')_2$ fragments to transfer protection. It was found that removal of the Fc portion of the antibodies by pepsin digestion completely abrogated the ability of these antibodies to confer passive protection. Thus, the divalent antigen-binding portion of the IgG was not sufficient to mediate protection, and it would appear that some effector mechanism(s) involving host effector components bearing Fc receptors is required for the immune elimination of schistosomes in this model. Examples of host factors that bear Fc receptors and are often implicated in immune effector mechanisms are complement, polymorphonuclear leukocytes, and macrophages. Complement may not be an important effector system in mice actively immunized with irradiated cercariae, however, since 1) mice genetically deficient in C5 develop the same level of immunity as their congenetically C5 normal counterparts,[13] and 2) the level of immunity in irradiated cercaria–immunized mice is not affected by decomplementation with cobra venom factor during the skin and/or lung phases of migration.[14] Likewise, eosinophils appear not to be required in this model, since ablation of eosinophil responses with an anti–interleukin-5 (IL-5) neutralizing monoclonal antibody failed to effect the level of protective immunity in irradiated cercariae–immunized mice.[15] Therefore, if we assume that our heterologous (rabbit-to-mouse) passive transfer system is analogous to the actively immunized mouse model, then the requirement for the Fc portion of IgG antibody may depend on Fc interaction with host components other than complement and eosinophils.

The finding presented in this report that high levels of protection can be passively transferred with IgG from rabbits multiply immunized with irradiated cercariae, along with previous demonstrations of passive transfer of protection with serum or immunoglobulin-fractions from immunized mice,[1, 12, 16] rats,[17–20] and rabbits,[2, 12] passive transfer of protection with monoclonal

antibodies,[21-26] and ablation of immunity in μ-suppressed mice[13] indicate a role for antibody in immunity to schistosomiasis. This does not preclude, however, that cell-mediated immune responses are also important in irradiated cercaria–induced immunity. Involvement of such responses is suggested by evidence of a role for L3T4$^+$ cells as determined by ablation experiments,[27,28] correlation of immunity in P/J and P/N mice to interferon-gamma (IFN-γ) production and macrophage activation,[29,30] and correlation of immunity with elevated levels of pulmonary T lymphocytes.[31] Thus, although passive transfer data demonstrate that specific cell-mediated responses are not always required in irradiated cercaria immunization models, cell-mediated elimination mechanisms may be important under some conditions. Indeed, a recent report suggests that Th1-associated responses, including IL-2 and IFN-γ production, predominate in mice singly immunized with irradiated cercariae, whereas Th2-associated responses, such as enhanced IgG1 production, are elevated in multiply immunized mice.[32]

In summary, it was demonstrated that 1) IgG antibodies from rabbits multiply immunized with irradiated cercariae transfer significant levels of protection to naive mice, 2) these antibodies are most effective when transferred at the time of lung phase migration, less effective when transferred at the time of challenge, and totally ineffective when transferred at the time of parasite residence in the liver, 3) the Fc portion of the IgG molecule is required for passive transfer of protection, and 4) the anti-KLH activity in irradiated cercaria–immunized rabbit serum is not necessary for passive transfer of protection, and high-titered Ig from a rabbit immunized with KLH does not passively transfer protection to mice.

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Authors' address: Beverly L. Mangold and David A. Dean, Schistosomiasis Research Laboratory, U.S. Naval Medical Research Unit No. 3, PSC 452, FPO AE 09835-0007.

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Mangold, Beverly L. and Dean, David A.

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Antibodies of the IgG subclass isolated from the sera of rabbits immunized with cercariae subjected to 50 Kilorads of gamma irradiation passively provided partial immunity against Schistosoma mansoni challenge in C57Bl/6J mice. These mice exhibited reductions in adult worm burdens of 43-61% compared with recipients of normal rabbit antibodies. Passively transferred IgG antibodies were most effective when given 4-7-days postchallenge; they were less effective when given just before challenge, and were totally ineffective when given 15 days postchallenge. It was also shown that the Fc portion of the IgG molecule was important for passive transfer of immunity. Finally, we observed that although some antibodies from irradiated cercaria-immunized rabbits recognized keyhole limpet hemocyanin (KLH), these KLH cross-reacting antibodies were not necessary for successful passive transfer of immunity. Antibodies from a KLH-immunized rabbit also failed to passively protect mice.

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